SHORT REPORT

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Potent Insecticidal Secondary Metabolites from the Medicinal Plant *Acanthus montanus*

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Abstract: *Acanthus montanus* (Nees) T. Anders. (Family: Acanthaceae) is a small shrub with sparse branches and soft stems, widespread in Africa, the Balkans, Romania, Greece and Eastern Mediterranean. Documented evidence showed that the leaves of the plant possess spasmolytic, analgesic, anti-inflammatory and antipyretic activities. In our ongoing research project; aimed at identifying new natural compounds with insecticidal activity, the alcohol extract of the aerial parts of *A. montanus* exhibited a significant activity against adult *Aedes aegypti*. Phytochemical study of the plant has resulted in isolation of nine compounds, eight of which exhibit variable degrees of insecticidal activity. *β*-sitosterol-3-O- *β* –D-glucoside (**1)** exhibited potent mosquitocidal activity (100% mortality) against adult *Aedes aegypti* at 1.25 µg/mg concentration, followed by palmitic acid **(2)** (90%), linaroside (**3)** (80%), and acetoside (**9)** (70%) respectively. It is noteworthy that this is the first report of insecticidal activity of *β*-sitosterol-3-*O*- *β* –D-glucoside, linaroside and acetoside.

Keywords: Acanthaceae**,** *Acanthus montanus*, adult *Aedes aegypti*, β-sitosterol glucoside, palmitic acid, protochatecuic acid, shikimic acid.

1. Plant Source

Acanthus montanus (Nees) T. Anders. (Family: Acanthaceae) is a small shrub with sparse branches and soft stems, widespread in Africa, the Balkans, Romania, Greece and the Eastern Mediterranean [1]. Aerial parts of the plant were collected from Cameroon in March 2003. Botanical identification was performed by Trish Flaster, the executive director of Botanical Liaisons, a voucher specimen, number ACM-2326, was deposited at the repository of National Center for Natural Products Research, School of Pharmacy, University of Mississippi, USA.

2. Previous Studies

Previous biological investigations have shown that; the leaves of *A. montanus* display spasmolytic, analgesic, anti-inflammatory and antipyretic activities [2-4]. In Cameroon the plant is used traditionally to treat various ailments namely; cough, carious teeth, pharyngitis, dysmenorrhoea, gastritis, false labour, epilepsy, and intestinal helminthiasis, in other regions of Africa it is used to alleviate uretheral discharge, chest pain, emesis, constipation, rheumatic pains, and syphilis [5-6].

Many species of genus Acanthus have been investigated and shown to contain several classes of secondary metabolites, particularly benzoxazinoides, phenylethanoides, lignans, flavonoides, megastigmanes, fatty acids and aliphatic alcohol glycosides [7-20]. Previous phytochemical studies of *A. montanus* reported the isolation of triterpenoid glycosides [21-22], phenyl ethanoides, bezoxazinoides and fatty alcohols [23]. However, no relevant literature, substantiating the evaluation of the insecticidal activity of the plant, has been reported.

3. Present Study

In our ongoing research project, aimed at identifying natural plant constituents with pesticidal activity [24], extracts of several plants were evaluated. The alcohol extract of the aerial parts of *A. montanus* displayed a significant activity against adult female *A. aegypti*, so it was chosen for the study. Aerial parts of the plant 400 g were exhaustively extracted with 80% MeOH, and the dried extract was subjected to several chromatographic techniques to yield nine compounds (**1-9)**. The isolated compounds were tested for insecticidal activity against *A. aegypti*, using the adult assay protocol established by Pridgeon *et al*., [25-26]. Detailed phytochemical and pharmacological procedures are provided as supporting information.

3.1. Identification of the isolated compounds.

On the basis of spectral data obtained from HR-ESI-MS and extensive NMR experiments (^{1}H, ^{13}C, APT, DEPT-135, COSY, HMQC, HMBC and NOESY) and by comparison with spectral values reported in the literature [27-33], the compounds obtained from *A. montanus* were identified as; *β*-sitosterol-3-O- *β* –D-glucoside (**1**), palmitic acid (**2**), linaroside (**3**), homoplantagenin (**4**), 5, 7, 3′-trihydroxy-6,4′ -dimethoxy flavone-7-O-glucoside(**5**), shikimic acid (**6**), protochatecuic acid (**7**), blepharin (**8**), and acetoside (**9**). It is noteworthy that this is the first report of isolation of (**1—7**) from *A. montanus*. The isolated compounds belong to different chemical classes; fatty acids, flavonoides, sterol glucoside, benzoxazinoide, phenolic acid, and phenyl ethanoid. Accordingly, the present work, confirms the typical profile of secondary metabolites found in the genus Acanthus. Moreover, it reports the first isolation of the two acids; protochatecuic and shikimic, from family Acanthaceae. Hence, adding another class of secondary metabolites not previously reported from this family. The occurrence of such metabolites provides a chemotaxonomic tool for further studies of this genus.

3.2. Insecticidal activity testing results

Eight of the isolated compounds were tested for activity against female adults of *A. aegypti*, at two concentrations; 1.25 µg/mg and 0.63 µg/mg. *β*-sitosterol glucoside (**1**) and palmitic acid (**2**), were the most active showing 100% and 90% mortality, respectively, at 1.25 µg/mg concentration, followed by linaroside (**3**) 80%, acetoside (**9**)70%, protochatecuic acid (**7**) 40%, and homoplantagenin (**3**) 30%. At concentration 0.63 µg/mg, compounds (**1**), (**2**), (**3**), and (**6**) were active showing 90%, 80%, 70%, and 10% adulticidal activity, respectively.

Acetone and Permethrin were used as negative and positive controls causing 0% and 100% mortality, respectively, at the tested concentrations. Permethrin $LD_{50} = 4.9 \times 10^{-5}$ [34].

The larvicidal activity of palmitic acid, against *Culex quinquefasciatus*, *Anopheles stephensi* and *A. aegypti*, was previously reported by Abdul-Rahman *et al*. 2000 [35], however, this is the first report of the adulticidal activity of these compounds.

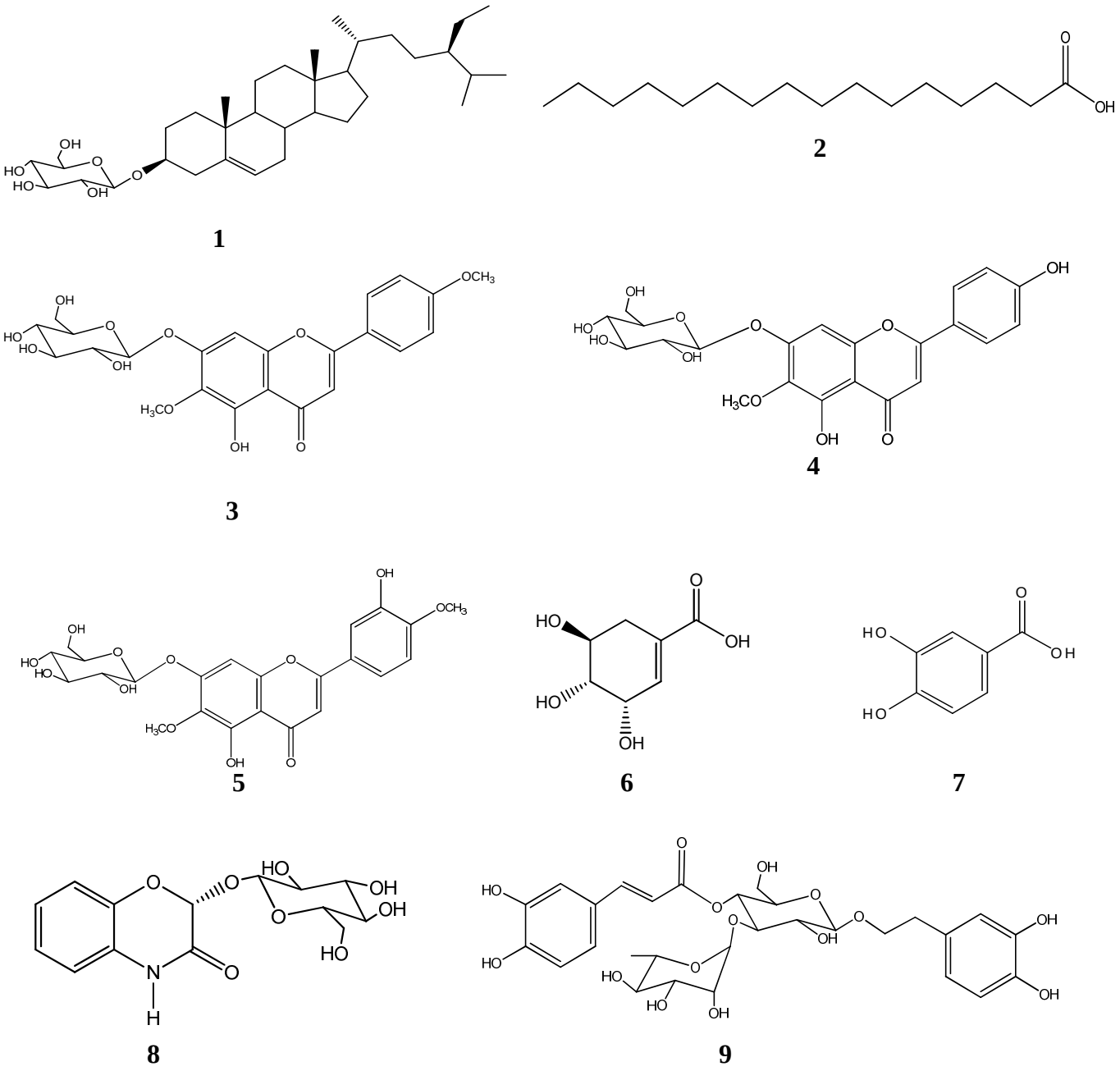

Figure 1: Compounds isolated from *Acanthus montanus*

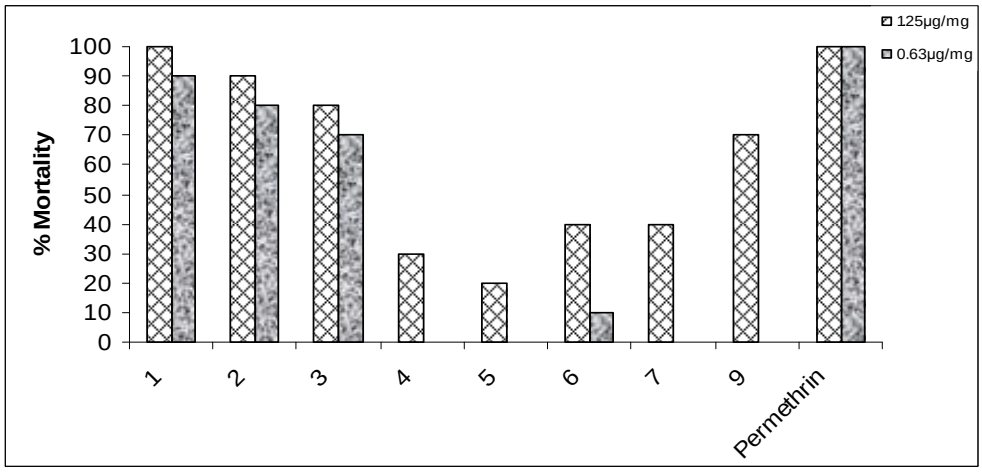

Figure 2: Mosquitocidal activity of isolated compounds

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/RNP

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